Taxonomic Status of Diploid Southern Hemisphere Brackens (*Pteridium*: Dennstaedtiaceae)

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Abstract

Diploid bracken ferns separate morphologically, genetically and reproductively into a predominantly northern hemisphere clade comprising 11 subspecies placed in *Pteridium aquilinum* (L.) Kuhn and a predominantly southern hemisphere clade of two taxa. Circumscription of *Pteridium esculentum* (G.Forst.) Cockayne is here emended to include both taxa of the southern clade, respectively *P. esculentum* subsp. *esculentum* from Australasia and *P. esculentum* subsp. *arachnoideum* (Kaulf.) J.A.Thomson comb. nov. from Central and South America.

Introduction

Recent reassessment of *Pteridium* Gled. ex Scop. confirms Tryon's (1941) basic concept of the genus as a well circumscribed entity separable into two groups of predominantly northern versus southern hemisphere distribution (Thomson 2000, 2004; Thomson & Alonso-Amelot 2002, Thomson et al. 2005, Thomson et al. 2008, Der et al. 2009). Tryon treated these two assemblages as subspecies of *P. aquilinum* (L.) Kuhn, including respectively 8 and 4 varieties. The taxa of predominantly northern hemisphere distribution are currently treated as a clade of African and Laurasian affinity (Der et al. 2009) referred to below as the 'northern' clade. This comprises 11 diploid ($2n = 104$) subspecies within *P. aquilinum* (for taxonomic summary see Table 1 in Thomson 2008; Marrs & Watt 2006).

Importantly, two of the four taxa placed by Tryon (1941) within his predominantly southern hemisphere subspecies, *P. aquilinum* subsp. *caudatum*, are now known to have arisen through hybridisation between elements of the southern and northern clades (Brownsey 1989, Thomson 2000). Both alloploid taxa are considered to be tetraploid, based on correlation of nuclear DNA content (Tan & Thomson 1990) with guard-cell length (Thomson 2000, Thomson & Alonso-Amelot 2002). One taxon occurs in Central and South America and is currently given full specific status as *P. caudatum* (L.) Maxon (= *P. aquilinum* var. *caudatum* (L.) Sadeb.) by Thomson & Alonso-Amelot (2002). The other alloploid taxon, found in Australasia and SE Asia, corresponds to Tryon's (1941) *P. aquilinum* var. *yarrabense* Domin. This is treated by Thomson & Alonso-Amelot (2002) as *P. semihastatum* (N.Wallich ex J.G.Agardh) S.B.Andrews pending the cytological investigation required to elucidate further the apparent variation in ploidy level and spore abortion noted amongst these plants by Brownsey (1989).

Two diploid ($2n = 104$) sister taxa comprise the remainder of Tryon's (1941) southern subspecies, forming an Australasian/South American pairing presumably of Gondwanan origin, referred to below as the 'southern' clade. These two taxa are given specific rank in most regional floras (see for example Brownsey 1989, 1998;

Mickel & Beitel 1988, Mickel & Smith 2004, Smith 1995) as *P. esculentum* (G.Forst.) Cockayne (= *P. aquilinum* var. *esculentum* (G.Forst.) Kuhn) of Australasia and *P. arachnoideum* (Kaulf.) Maxon (= *P. aquilinum* var. *arachnoideum* (Kaulf.) Brade) of Central and South America.

Separation of Southern and Northern Clades

The finding that hybrids between diploid northern and southern hemisphere progenitors may show significant spore abortion leading to partial or complete sterility suggests some degree of genetic incompatibility between the parent species (Brownsey 1989). Earlier, Klekowski (1973) attempted a direct experimental assessment of interfertility between representatives of these lineages using mass-cultured gametophytes in vitro, but unfortunately his results are difficult to interpret. Crosses between *P. aquilinum* subsp. *decompositum* (Gaud.) Lamoureux ex J.A.Thomson from Hawaii and 'arachnoideum' (presumably 2n = 104, see Postscript p. 479 in Jarrett et al. 1968) from the Galapagos Islands produced no viable sporophytes, suggesting possible reproductive barriers. However, in contrast, crosses involving gametophytes of *P. aquilinum* subsp. *decompositum* from Hawaii with *P. caudatum* from Honduras and 'arachnoideum' from Peru both produced viable sporophytes, as did crosses of Galapagos 'arachnoideum' with both Honduran *P. caudatum* and Peruvian 'arachnoideum'. This complex pattern was interpreted by Klekowski as indicating "genetic endemism" of the Galapagos 'arachnoideum'. However, because *P. caudatum* carries genomes from both northern and southern lineages, these results may be explicable in terms of reproductive discontinuity between diploid northern and southern clades if the Peruvian 'arachnoideum' used by Klekowski also carried genomic elements from the northern 'aquilinum' lineage as a result of past introgression. Unfortunately, no voucher is cited by Klekowski (1973) and the identity of the Peruvian spore-parent used for the crosses cannot be verified. Both 'arachnoideum' and *P. caudatum* occur in Peru (e.g. NSW360578, *C.M. Belshaw 3449*, Lamas, Peru, is annotated "Det. Rolla Tryon 1967 New to Peru"). Sporelings from the crosses made by Klekowski were not grown on, so that their viability to maturity and their fertility are unknown.

Many lines of evidence highlight divergence of the northern and southern bracken lineages, while linking 'esculentum' and 'arachnoideum' as sister taxa in the southern clade. Morphological characters following this pattern include the presence versus absence of (i) free laminal lobes on segment axes, (ii) gnarled intervein trichomes on the lower laminal surfaces (Thomson & Martin 1996), and (iii) well-developed petiolar roots (O'Brien 1963, 1990; McGlone et al. 2005). Nuclear genome sizes for 'esculentum' (2C = 16.0 ± 1.0 pg DNA) and 'arachnoideum' (2C = 16.5 ± 1.0 pg DNA) are comparable, but differ significantly from those measured in four subspecies of *P. aquilinum* (range 2C = 18.9 to 19.8 ± 1.0 pg DNA; Tan & Thomson 1990). A restriction site polymorphism common to both 'esculentum' and 'arachnoideum' that is not present in northern taxa has been identified in 45S rDNA (summarised in Thomson 2004; Thomson et al. 1995). The chloroplast genomes of the two southern taxa share a common sequence (Haplotype C, Thomson et al. 2005, Der et al. 2009) in the *rps*4 to *trn*S region distinct from the corresponding haplotypes of the northern lineage. A sequence comprising 245 bp in *mt-ITS* shows 8 unambiguous differences between *P. aquilinum* from Britain and 'esculentum' from Australia (Wolf 1996).

Molecular data calibrated using the fossil record place the divergence of *Pteridium* and *Dennstaedtia* at between 114 Myr (Schneider et al. 2004) and 92 Myr ago (Pryer et al. 2004). Cladistic and phenetic analyses utilising both nuclear (Thomson et al. 1995, see Fig. 3A; Thomson 2000, Thomson et al. 2005) and chloroplast sequence-related data (Tan 1991, Der et al. 2009) show basal divergence of diploid bracken taxa into the northern and southern clades that may date to separation 100 to 30 Myr ago of Africa from a landmass including Australia, Antarctica, South America and New Zealand (Der et al. 2009, McLoughlin 2001, Scotese 2001). Even before the appearance of ocean barriers, strong latitudinal climatic influences within Gondwana are likely to have affected plant distribution (McLoughlin 2001) and may have facilitated differentiation of the two bracken clades. The close relationship of 'esculentum' and 'arachnoideum' presumably reflects their continued contact at least until break up of the contiguous East Gondwanan landmasses representing South America, Australia and New Zealand. This separation culminated in the opening of the Drake Passage 32–28 Myr ago, and established the South Circumpolar Current that hastened the cooling of Antarctica (McLoughlin 2001).

Taxonomy of the Southern Clade

The distinction between southern hemisphere brackens and northern taxa assigned to *Pteris aquilina* L. was first emphasised by Hooker (1858). He delimited var. *esculenta* "which inhabits principally New Zealand, Australia, the Polynesian Islands and tropical South America" as comprising three forms, These were later referred by Tryon (1941) to *Pteridium aquilinum* var. *yarrabense*, var. *esculentum*, and var. *arachnoideum*. The latter two of these southern taxa are diploid and are both treated here as subspecies of *P. esculentum*.

Pteridium esculentum (G.Forst.) Cockayne, *Rep. Bot. Survey Tongariro Natl Park* 34 (1908), emend. J.A.Thomson

Basionym: *Pteris esculenta* G.Forst., *Pl. Esc.* 74 (1786).

Lectotype (designated by Brownsey 1989): *Forster Herbarium 258 (418)*, BM001048405.

Type locality: New Zealand.

Description: Terrestrial fern with long creeping underground rhizomes. Fronds variable in size, c. 0.25–2.0 m long. Stipe c. 5–10 mm diam., bearing petiolar roots at base above junction with rhizome. Hairs present on fronds and rhizomes, scales absent. Nectaries present at bases of pinnae. Vernation gradual, acropetal. Laminae broadly elliptic to broadly ovate-triangular, 3- or 4-pinnate. Free lobes forming narrow wings, or long-decurrent segment bases present on segment axes. Ultimate segments linear, acute, entire, adnate and decurrent on one side. Laminal intervein surfaces with minute gnarled (distorted, irregularly tuberculate) trichomes (mostly 0.05–0.15 mm in length but occasionally longer, especially close to veins) causing a mealy appearance at low magnification (absent in a few glabrous or extreme shade-forms). When fertile, sori more or less continuous around margin of ultimate segments, protected by a membranous false indusium formed by the reflexed segment margin. Spores trilete, irregularly granulate.

Chromosome number: 2n = 104 (references in Brownsey 1989)

Genome size: 2C *circa* 16 pg DNA (Tan & Thomson, 1990).

Distinguishing features: *P. esculentum* is distinguished from *P. aquilinum* in having free lobes (or less commonly long-decurrent segment bases) on pinna and pinnule axes, well developed petiolar roots at the base of the stipe above its junction with the rhizome (see Fig. 1 in O'Brien 1990; O'Brien1963, McGlone et al. 2005), gnarled trichomes on lower laminal intervein surfaces (Thomson & Martin 1996) causing a 'farinaceous' (Tryon 1941) or 'mealy' (Brownsey 1989) appearance, and by its smaller genome size (2C *circa* 16 pg versus *circa* 19 pg DNA, Tan & Thomson 1990).

Distribution: Australasia, Central and South America.

Notes: petiolar roots are usually present, often numerous and strongly developed, in subspp. *esculentum* and *arachnoideum*, sometimes present in *P. caudatum* and *P. semihastatum*. Petiolar roots rarely occur in subspecies of *P. aquilinum* but when present are few and sparse (see for example subsp. *pseudocaudatum* (Clute) Hultén, NSW362481, *R. Dale Thomas 94849*, 01 Nov 1985, Arkansas, USA; subsp. *japonicum* (Nakai) A.Löve & D.Löve, NSW420866, *Wu Shifu 44*, 05 Jun 1981, Lingan Hsien, China).

Pteridium esculentum (G.Forst.) Cockayne subsp. ***esculentum***

Synonymy: *Pteridium aquilinum* (L.) Kuhn var. *esculentum* (G.Forst.) Kuhn, *Chaetopt.* 27 (1882), and see Tryon (1941).

Distinguishing features: *P. esculentum* subsp. *esculentum* differs from *P. esculentum* subsp. *arachnoideum* in that segment midribs are slightly flattened to terete rather than winged (bilaterally sulcate against the lower segment surface), midribs and main veins of the ultimate segments are appressed-pubescent rather than finely arachnoid-pubescent with white hairs, and the margin of the false indusium is usually entire and glabrous rather than erose and sparsely ciliate.

Distribution: Australia, New Zealand, New Caledonia, Solomon Islands, New Guinea, Solomon Is, Vanuatu, Fiji, Tonga; see Brownsey (1989).

Ecology: see O'Brien (1963), McGlone et al. (2005).

Notes: petiolar roots are well exemplified by NSW361591 (*R. Melville 3843 & J.H. Willis*, 11 May 1953, Victoria, Australia).

Pteridium esculentum (G.Forst.) Cockayne subsp. ***arachnoideum*** (Kaulf.) J.A.Thomson, **comb. nov.**

Basionym: *Pteris arachnoidea* Kaulf., *Enum. Filic.* 190 (1824) nom. cons. prop. (Schwartzburd & Prado 2011).

Type: L.A. von Chamisso s.n., LE.

Type locality: Brazil

Synonymy: *Pteridium arachnoideum* (Kaulf.) Maxon, *J. Wash. Acad. Sci.*14:89 (1924), and see Mickel & Smith (2004); *Pteridium aquilinum* (L.) Kuhn var. *arachnoideum* (Kaulf.) Brade, *Z. Deutsch. Verein Wiss. Kunz São Paulo* 1:56 (1920), and see Tryon (1941).

Distinguishing features: separated from subsp. *esculentum* by the covering of fine, often twisted, white arachnoid hairs on the segment midribs and main veins which are slightly flattened and bilaterally sulcate, giving a winged rather than terete appearance, and by the erose margin of the false indusium.

Distribution: Central America from Mexico south including the Caribbean Is, South America except Chile (Smith 1995, Mickel & Beitel 1988, Mickel & Smith 2004, Ortega 1990).

Ecology: see Alonso-Amelot & Rodulfo-Baechler (1996), Portela et al. (2008), Silva & Matos (2006).

Notes: the indumentum of the lower laminal surface of midrib, vein and intervein surfaces in subsp. *arachnoideum* is particularly variable. Hairs of veins and of the intervein surface appear to be under separate, although interacting, morphogenetic control in *Pteridium*, and either or both may be glabrous in some specimens of subsp. *arachnoideum* (Thomson & Martin 1996). For example, typical relatively long, twisted, thin, white arachnoid hairs of the veins of the ultimate segments in subsp. *arachnoideum* overlie the gnarled trichomes of the intervein areas in NSW361275 (*F.J. Ortega 3389*, 19 Jan 1993, Venezuela) but these arachnoid vein hairs are absent in NSW360580 (*K.U. Kramer 3050 & W.H.A. Hekking*, 26 Feb 1961, Surinam; compare Figs 5 E,F in Thomson & Martin 1996). Petiolar roots are evident in NSW 362662 (*E.R. Rosenstock 267*, 06 Apr 1914, Brazil).

Discussion

Morphological, molecular and reproductive evidence of the basal separation of *Pteridium* into a northern clade of African/Laurasian distribution and a southern Australasian/South American clade has been reviewed above. This dichotomy reflects the single major discontinuity in the genus, recognised in current taxonomic treatments by separation of the northern forms into *P. aquilinum* (see for example, Thomson 2004, 2008) and here by grouping the two southern forms within *P. esculentum*. Consistency of ranking is maintained across the genus by this nomenclatural change, and it focuses attention on significant hierarchical information about relationships that is hidden if the two southern taxa are each given full specific status.

Objective assessment of infra-specific taxonomic ranking is often difficult, especially when dependent mainly on morphological evidence. Phenetic or cladistic analysis based on the use of amino acid or nucleotide sequence data to generate measures of similarity provides one useful approach. In such cases, nodes are positioned on an indirectly time-related scale that reflects cumulative mutational change, permitting meaningful comparison within and between phenograms (Avise 2009). This approach has been particularly helpful in *Pteridium*, where an attempt has been made to standardise subspecies ranking in the northern clade covering *P. aquilinum* (Thomson, 2004, Thomson et al. 2005, Thomson et al. 2008). An example is provided by DNA-fingerprinting data for 'esculentum', 'arachnoideum', and representatives of 10 of the 11 subspecies of *P. aquilinum* comprising the northern clade (see Fig. 6 in Thomson et al. 2005). Arbitrarily-Primed PCR and Inter Simple Sequence Repeat data for 41 primers allowed band presence/absence to be scored for 475 sites and used to calculate similarity matrices for clustering analysis. The northern and southern clades separated basally at a coefficient of association (CA) of 0.52, considered here to be consistent with the species ranking suggested by reproductive discontinuity between these two clades. All 10 of the northern taxa considered to be of subspecies status on morphological and inter-breeding criteria (Thomson 2004, 2008) separated from sister clades between CA = 0.65 and CA = 0.75. The two southern taxa, 'esculentum' and 'arachnoideum', separate at CA = 0.82 consistent with subspecies rather than species ranking.

The case for assigning 'arachnoideum' to subspecies level as *P. esculentum* subsp. *arachnoideum* is clear and strong. The close relationship of 'arachnoideum' and 'esculentum' is supported by the distinctive synapomorphy of gnarled intervein trichomes, and by the extensive molecular data summarised above. They share the distinctive plesiomorphy (with respect to *Dennstaedtia*) of free laminal lobes on segment midribs. Subspecies ranking of these two southern taxa maximises the information provided in the systematic treatment of the genus as a whole, and establishes consistent infra-generic rankings across *Pteridium*. Importantly, the name 'arachnoideum' is retained, albeit at lower rank than recommended by Schwartsburd & Prado (2011) in their recent conservation proposal.

Acknowledgments

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